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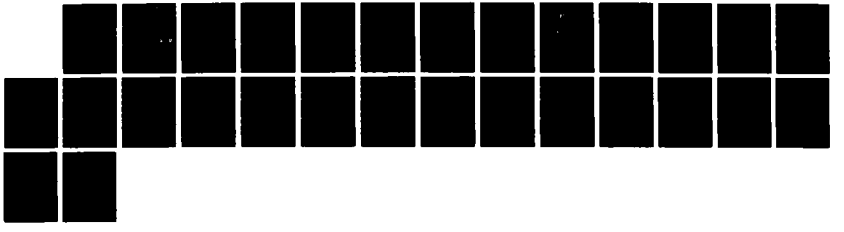

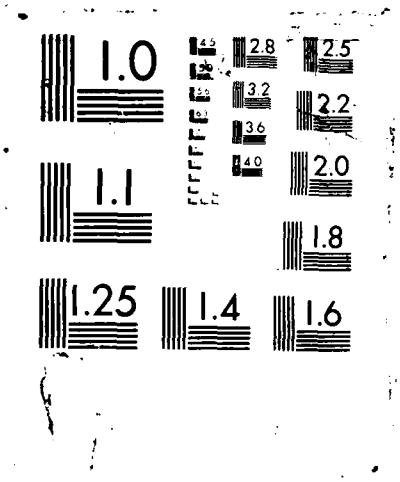
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

During the second year of this contract, we have purified human erythrocyte acetylcholinesterase to >87% homogeneity by MAb affinity chromatography and size-exclusion HPLC and have obtained sequence data for >100 residues, derived from tryptic, chymotryptic, and V8 peptides. We have used these sequence data to synthesize oligonucleotide probes with which to screen two human cDNA libraries for the gene encoding human AChE. In addition, we have identified a large number of MAb that bind to acetylcholinesterase and we have begun to characterize several of these antibodies that are capable of inhibiting acetylcholinesterase.

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Figure 10: Effect of pre-incubation of acetylcholine with neostigmine on the binding of monoclonal antibodies to the enzyme

Figure 11: Effect of pre-incubation of acetylcholine with BW 284c51 on the binding of monoclonal antibodies to the enzyme

Figure 12: Effect of pre-incubation of acetylcholine with edrophonium on the binding of monoclonal antibodies to the enzyme

REPORT

Problem

The goals of this research are to biochemically and immunochemically characterize the molecular structure of the active site of human acetylcholinesterase (AChE). This information should lead to the capability of chemically or immunologically synthesizing analogues of the esteratic site. The first step or problem in this program has been the purification of the enzyme from human erythrocytes in preparative quantities. Our research during the second year has been directed primarily toward using our highly purified AChE for (1) the production of AChE-specific monoclonal antibodies and (2) amino acid sequence determination and synthesis of oligonucleotide probes to allow us to clone the gene encoding the AChE molecule.

Background

AChE is associated chiefly with cells involved in cholinergic synaptic transmission and is also found in a few non-neuronal cells like erythrocytes. Fambrough, et al. (Proc. Natl. Acad. Sci., USA 79, 1078-1082, 1982), prepared five monoclonal antibodies that bound to purified human erythrocyte AChE, each reacting with different antigenic sites on the AChE molecule. All of these antibodies cross-reacted with human and monkey neuromuscular junctions. It was concluded that a high degree of homology exists between the AChE of erythrocytes and neuromuscular junctions.

The most available source of human AChE is the erythrocyte. AChE is an integral part of the red blood cell membrane (Biochem. Biophys. Res. Commun. 39, 267-273, 1970; Biochem, Biophys., Acta 255, 251-272, 1971), and is one of the most active of known catalytic agents, with a turnover number of about 6×10^5 moles/min/active site. Purification of human erythrocyte AChE has been reported by several workers (Biochem. Biophys. Acta 370, 468-476, 1974; Eur. J. Biochem. 57, 469-480, 1975; J. Biol. Chem. 259, 5643-5652, 1984.)

Herein we report the purification of human erythrocyte AChE by monoclonal antibody chromatography and the use of this purified AChE for amino acid sequence determination and generation of enzyme-specific monoclonal antibodies.

A. PURIFICATION OF HUMAN ERYTHROCYTE ACETYLCHOLINESTERASE:

I. Monoclonal Antibody Affinity Chromatography

We have employed monoclonal antibody affinity chromatography for simple and efficient purification of the erythrocyte enzyme to near homogeneity with high yield (Table 1). Approximately 500 μg is obtained from 1000 ml of packed red blood cells. This yield is similar to that reported by Rosenberry and Scoggin with the acridinium ligand procedure (J. Biol. Chem. 259, 5643-5652, 1984). Recovery of enzyme activity is about 15 percent with a purification of more than 130,000-fold. The yield of AChE protein is about 60 percent, as indicated by radioimmunoassay of enzyme protein, indicating that the overall purification of AChE is about 500,000-fold.

An important modification over earlier procedures was the replacement of Triton X-100 used to solubilize the enzyme by β-D-octylglucoside during affinity chromatography and elution from the column at pH 10.5. Enzyme elution in the presence of Triton X-100 and higher pH apparently caused significant aggregation of the molecule. The change of detergent and pH results in greater yield and purity of material.

TABLE 1: Purification of human erythrocyte acetylcholinesterase[a]

Purification Step	Volume (ml)	Total Protein[b] (mg)	Total Activity[c] (ΔA/min)	Activity Recovery (%)	Specific Activity (ΔA/min/mg)	Fold Purification
HEMOLYSATE	21,217	488,286	262,810		0.5	1
RED CELL GHOSTS	1,132	5,040	245,608	93	52	100
100,000 x g SUPERNATANT	1,027	994	165,671	63	178	342
ANTIBODY AFFINITY	0.9	0.7	34,597	13	68,180	131,115

[a] Results are the mean value of four purifications.
[b] Protein was measured by the modified method of Lowry et al. (J. Biol. Chem. 193, 265-275, 1951).
[c] Enzyme activity was measured as described by Ellman et al. (Biochem. Pharm. 7, 88-95, 1961).

II. Size-exclusion HPLC

Final purification of the affinity isolated material is achieved by size exclusion HPLC chromatography. AChE elutes as a single peak of enzymatic activity and protein. The enzyme is recovered as a high M_r fraction (>232,000 Da) rather than at the 140,000 Da position of the dimeric protein; we attribute this effect to the detergent bound to the protein.

The HPLC step is important to subsequent sequence studies as it removes low molecular weight contaminants that possibly could be present in high molar concentration, even when insignificant in mass or absorbance at 280 nm.

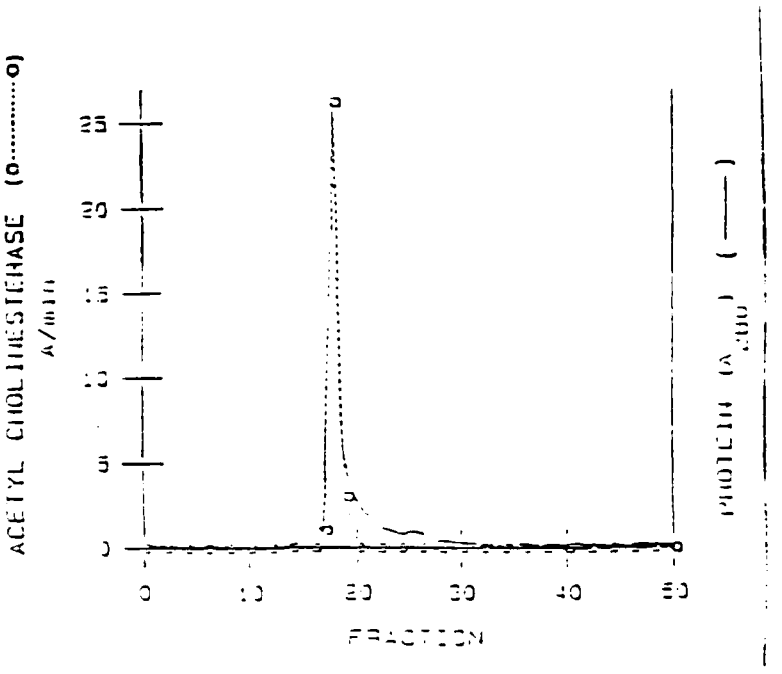

Figure 1: HPLC size exclusion chromatography of purified AChE. The concentrated affinity-purified fraction of AChE (20 µl, 10 µg) was placed in a 7.5 mm X 30 cm TSK-SW3000 column. The column was eluted with 50 mM $NaPO_4$, pH 7.5, 150 mM NaCl, 0.3 percent octylglucoside. The fraction volume was 0.5 ml. The tracing is absorbance at 280 nm with a setting of 0.02 absorbance units full scale. The peak fraction corresponded exactly to enzyme activity and to radioactivity of ^{125}I-AChE (0.01 µg, 1.3 X 10^6 cpm) added as an internal marker.

III. Purity

AChE purity was analyzed by SDS-PAGE (Fig. 2). The protein was stained by silver nitrate reduction. The AChE was apparently over 90 percent pure following affinity chromatography and appeared to be >98 percent pure after HPLC. The enzyme was present as a dimer before disulfide reduction and as a 70,000 Da monomer after reduction. When labeled with [^{3}H]OFP, a single band corresponding to the enzyme was observed.

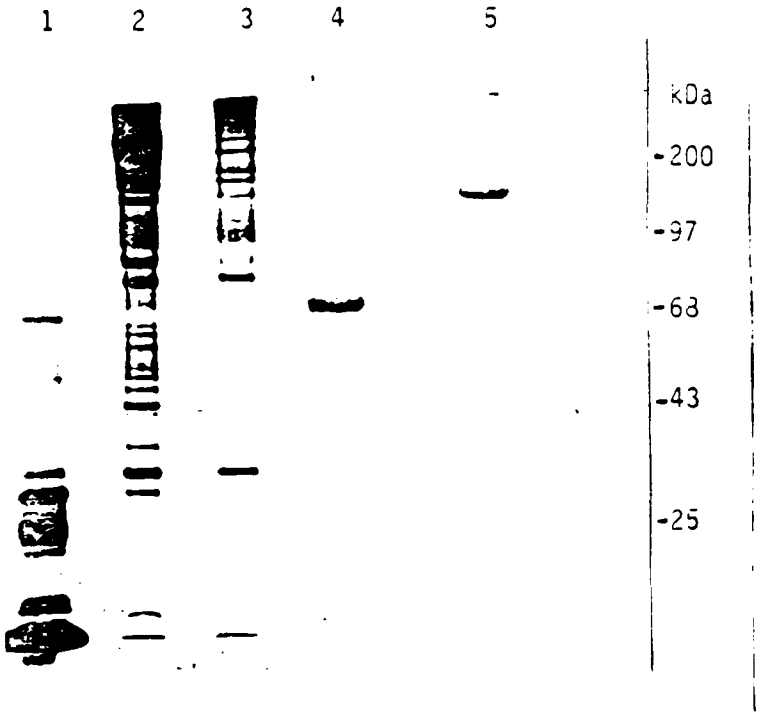

Figure 2: SDS-PAGE analysis of AChE purification. Lane 1, hemolysate, 60 µgm; lane 2, red blood cell ghosts, 10 µg; lane 3, 100,000 xg supernatant, 3 µgm; lane 4, affinity purified AChE, 1.5 µg after disulfide reduction; lane 5, affinity purified AChE, 1.5 µgm protein, without disulfied reduction. The protein bands were visualized by silver staining. M_r standards are myosin heavy chain, 200 kDa; phosphorylase b, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; chymotrypsinogen, 25 kDa.

IV. Two-dimensional electrophoresis

The purified proteins contained several isoelectric varients, typical of glycoproteins with charge heterogenity in the oligosaccharides of the molecule (Fig. 3).

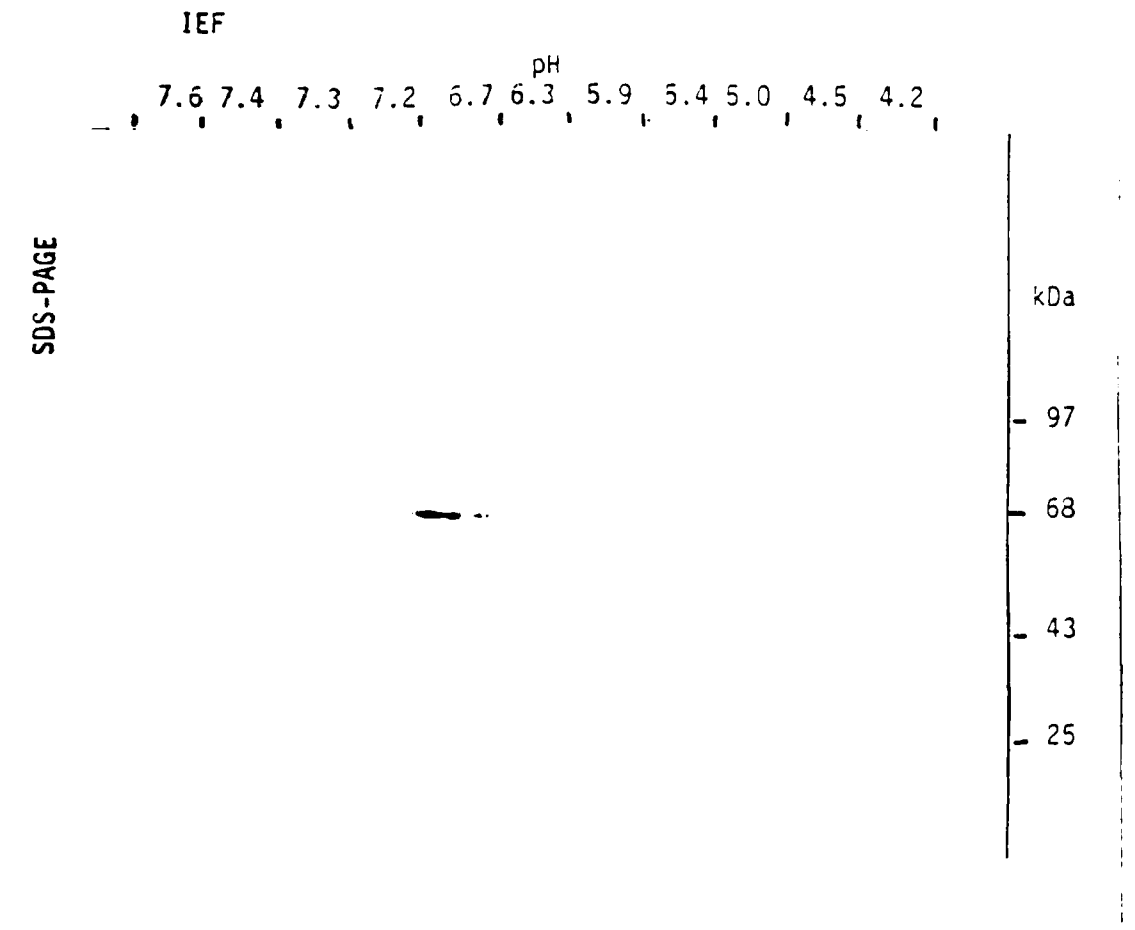

Figure 3: Two-dimensional gel analysis of purified AChE. HPLC purified AChE, 7 ug, was analyzed as described by O'Farrell (J. Biol. Chem. 250, 4007-4021, 1975). The protein was stained with silver nitrate. M_r standards on the left are phosphorylase b, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; and chymotrypsinogen, 25 kDa.

B. AMINO ACID SEQUENCE ANALYSIS:

We currently have sequence data for >100 residues, obtained from tryptic, chymotryptic and V8 peptides. These peptides represent approximately 20 percent of the intact enzyme molecule. The sequence data is summarized in Tables 2-5.

Sequences were obtained with the vapor phase protein sequencer (Model 470A, Applied Biosystems) described by Hewick et al. (J. Biol. Chem. 256, 7990-7997, 1981).

I. Tryptic peptides

Peptide mixtures generated by trypsin digestion were separated by reverse-phase HPLC. Approximately 23 peptides were resolved (Fig. 4). At this time, 5 have yielded satisfactory sequence data (Table 2).

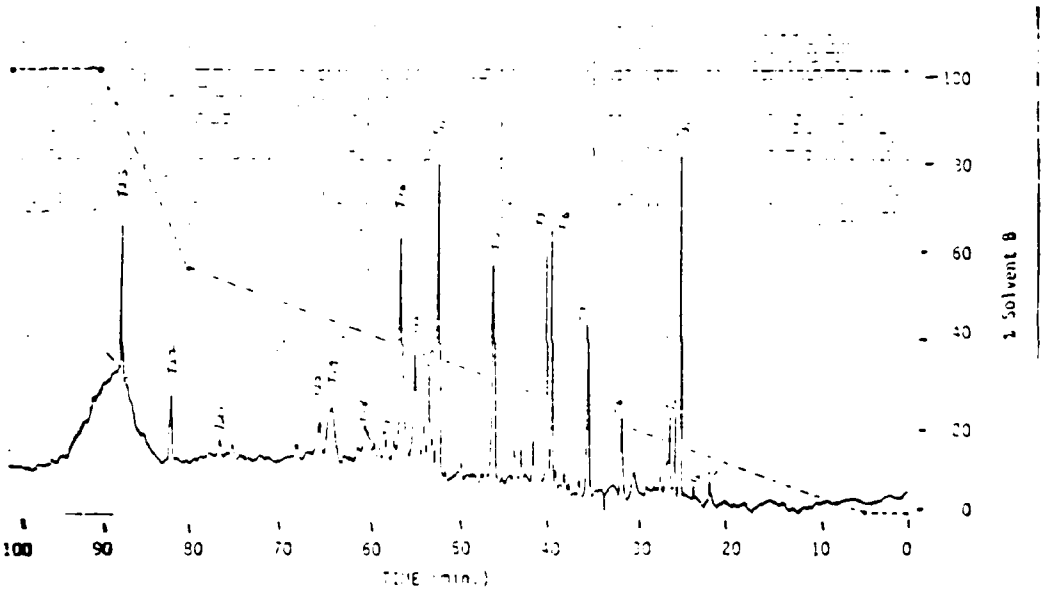

Figure 4: Preparation of tryptic peptides of AChE. AChE (70 µg) in 2 ml of 20 mM $NaPO_4$, pH 7.0, containing 0.2 percent octylglucoside was treated at room temperature for 8 h with a total of 3 µg of trypsin. The digest was applied to a Vydac C4 column (4.6 mm X 25 cm), maintained at 45 °C. The solvents were (A) 0.1 percent trifluoroacetic acid (TFA) and (B) acetonitril:propanol (2:1) containing 0.1 percent TFA. The peptides were eluted at a flow rate of 1 ml/min with the following gradient program: 0 percent B for 5 min, 0 percent B to 55 percent B for 75 min, 55 percent B to 100 percent B for 10 min, 100 percent B for 10 min. The fractions were dried under vacuum and subjected to amino acid sequence analysis.

Table 2. Sequence analysis of AChE tryptic peptides

Cycle	T7			T9		T10			T11			T16	
	Amino acid	Yield (1)	Yield (2)[a]	Amino acid	Yield	Amino acid	Yield (1)	Yield (2)[a]	Amino acid	Yield (1)	Yield (2)[a]	Amino acid	Yield
		pmole	pmole		pmole		pmole	pmole		pmole	pmole		pmole
1	Ala	X[b]	80	Thr	99	X	X	X	Val	216	173	X	X
2	Val	224	46	Arg	50	Pro	276	298	Gly	142	214	X	X
3	Leu	265	62	Pro	210	Gln	116	108	Val	202	175	Thr	28
4	Gln	188	57	X	X	Try	156	24	Pro	296	189	Leu	75
5	Ser	56	X	Gln	73	Pro	218	237	Gln	119	64	Asp	32
6	Gly	129	20	Val	118	Pro	225	253	Val	182	101	Val	19
7	Ala	176	26	Leu	113	Tyr	144	148	Ser	40	12	Pro	49
8	Pro	190	57	Val	118	Thr	71	62	Asp	56	43	Leu	50
9	Asn	85	X	X	X	Ala	158	132	Leu	160	120	X	X
10	Gly	124	19	His	25	Gly	141	81	Ala	117	82	Met	36
11	Pro	131	35	Glu	71	Ala	101	99	Ala	126	90	Gly	27
12	Try	46	17	Asp	64	Gln	132	82	Glu	X	92	Val	33
13	Ala	103	12	His	25	Gln	122	105	Ala	106	64	Pro	37
14	Thr	35		Val	85	Tyr	116	95	Val	110	84	X	X
15	Val	82		Leu	94	Val	125	100	Val	92	108	Gly	25
16	Gly	101		Pro	98	Ser	26	18	Leu	115	108	Tyr	15
17	Met	74		Gln	48	Leu	93	116	Asp	X	22		
18	Gly	44		Glu	50	Asp	36	29	Tyr	73	59		
19				Asp	59	Leu	75	63	Thr	29	X		
20				Val	20	Arg	34	38	Asp	29	12		
21				Phe	45	Pro	85	94	Try	36	X		
22						Leu	63	42	Leu	64	47		
23						Glu	48	32	X	X	X		
24						Val	49	44	Pro	60	X		
25									X	X	X		
26									Asp	16	X		
27									Pro	46	X		
Avg. repetitive yield (%)		88.35		86.5	88		89.7	80.2		95.2	83		86

[a] Repeated analysis
[b] Value not identified

II. Chymotryptic peptides

Over 40 fractions have been obtained from AChE treated with chymotrypsin (Fig. 5). The amino acid sequence of each of these peptides currently is being analyzed. The sequence of three of these peptides in shown in Table 3.

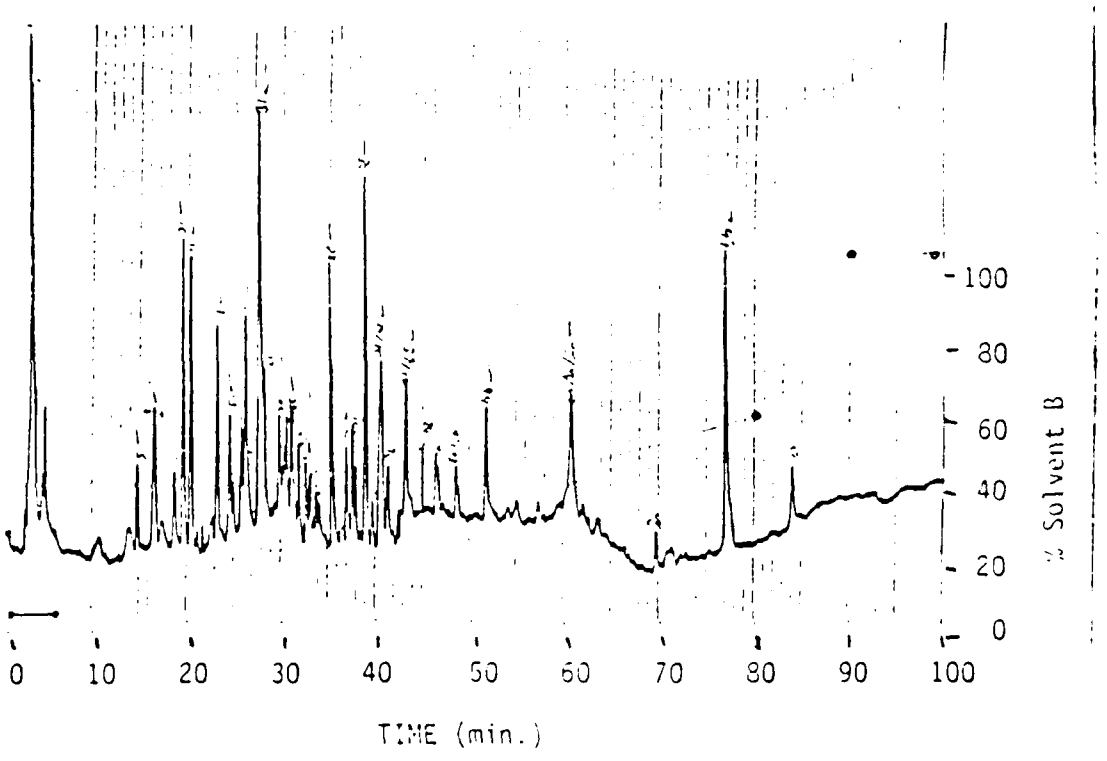

Figure 5: Preparation of chymotryptic peptides of AChE. AChE (150 ugm in 350 ul 100 mM sodium phosphate, pH 8.0 containing 0.5 percent octylglucoside) was treated at 37°C for 2 h with 3 ugm of chymotrypsin. The peptides generated were separated on a C4 Vydac reverse phase column as described for tryptic peptides.

Table 3. Amino acid sequence analysis of chymotryptic peptides of AChE

	CH_1		CH_2		CH_3	
Cycle	**Amino acid**	**Yield**	**Amino acid**	**Yield**	**Amino acid**	**Yield**
		pmole		pmole		pmole
1	Ala	494	X	X	X	X
2	Ala	426	Asn	248	X	X
3	Gln	290	Gly	231	Try	93
4	Gly	368	Ala/Glu	148/137	Gly	36
5	Ala	346	Phe	106	Met	34
6	Arg	69			Gly	57
7	Val	355			Glu	17
8	Tyr	125				
9						
10						

III. V8 peptides

Digestion of AChE with S. aureus V8 protease yielded 9 peptide fractions upon resolution on size exclusion HPLC (Fig. 6). Two of these peptides (V3 and V6) have been analyzed (Table 4) and contain sequences highly similar to sequences within tryptic peptides T9 and T11 (Table 5). Repeated sequencing of these fragments is required to determine if the differences (residues 1 and 2 of V3 as compared to T9 and residues 5 and 7 of V6 as compared to T11) are significant.

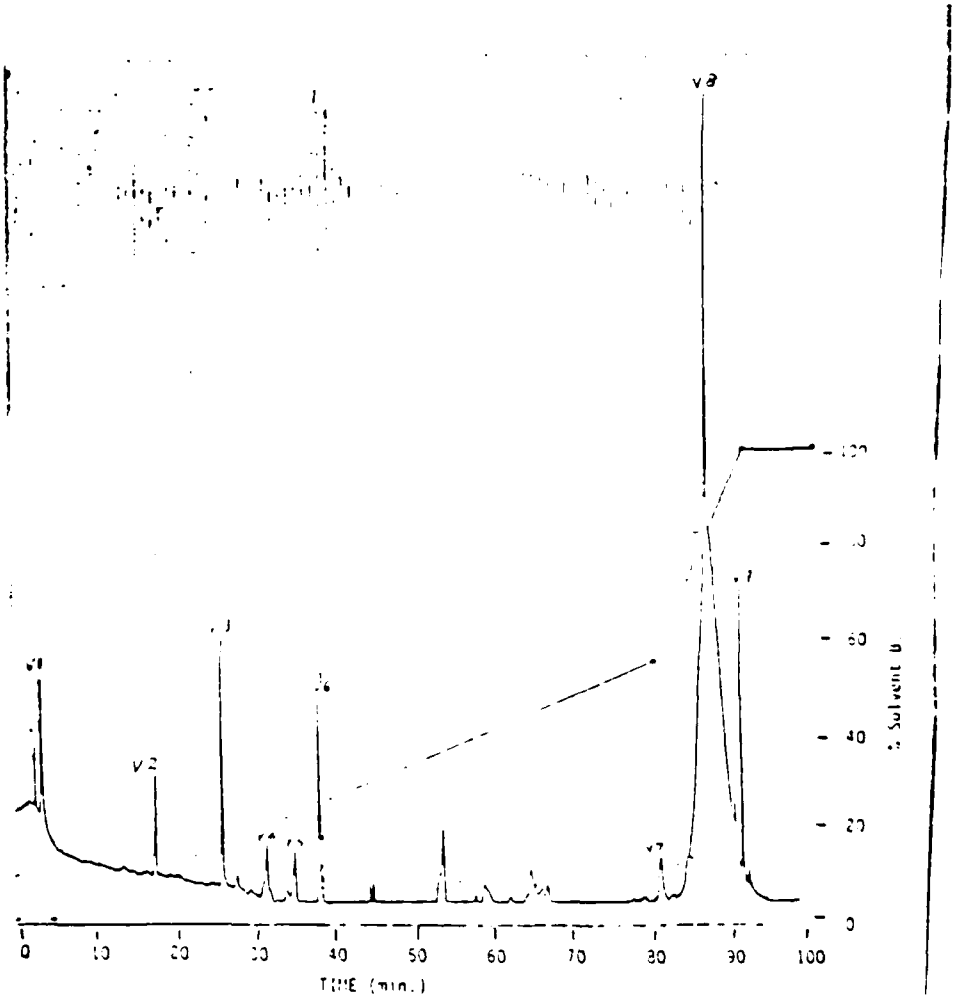

Figure 6: Preparation of V8 peptides of AChE. The AChE (100 ugm) purified by size exclusion HPLC was mixed with 3 ugms of S. aureus V8 enzyme in 0.2 M sodium phosphate buffer pH 8.2 containing 0.2 percent octylglucoside. The mixture was incubated at 37°C for 18 h. The peptides were then separated on a C4 vydac reverse phase column under the conditions described for the separation of tryptic peptides.

Table 4. Sequence analysis of AChE V8 peptides

Cycle	V3		V6	
	Amino acid	Yield	Amino acid	Yield
		pmole		pmole
1	Try	79	Ala	156
2	X		Val	114
3	Val	53	Val	117
4	Leu	58	Leu	123
5	Pro	82	Ala	27
6	Gln	30	Tyr	67
7	Glu	13	Leu	31
8			X	
9			Try/Ala	27/24
10			Leu	47
11			Leu	44
12			Pro	21
13			Gly	11
14			Asp	5
15			Pro	44
16			Ala	21
17			Gly	7
18			Leu	50
19			Gly	9
Repetitive yield				66 (%)

Table 5. Comparison of tryptic acid and V8 peptides

	T9		V3		T11		V6
1	Thr				Val		
2	Arg				Gly		
3	Pro				Val		
4	X				Pro		
5	Gln				Gln		
6	Val				Val		
7	Leu				Ser		
8	Val				Asp		
9	X				Leu		
10	His				Ala		
11	Glu				Ala		
12	Asp	1	Try		Glu		
13	His	2	X		Ala	1	Ala
14	Val	3	Val		Val	2	Val
15	Leu	4	Leu		Val	3	Val
16	Pro	5	Pro		Leu	4	Leu
17	Gln	6	Gln		Asp	5	Ala
18	Glu	7	Glu		Tyr	6	Tyr
19	Asp				Thr	7	Leu
20	Val				Asp	8	X
21	Phe				Try	9	Try/Ala
22					Leu	10	Leu
23					X	11	Leu
24					Pro	12	Pro
25					X	13	Gly
26					Asp	14	Asp
27					Pro	15	Pro
						16	Ala
						17	Gly
						18	Leu
						19	Gly

IV. N-terminal sequence analysis

We have attempted N-terminal sequence analysis of AChE five times. The results have been ambiguous with inconsistant results and mixed signals at certain residues. We did not repeat the sequence reported previously in abstract form.

The 70-kDa subunits possibly differ. Haas and Rosenberry (Anal. Biochem. 148, 154-162, 1985) have reported the presence of 2

N-terminal amino acids with stoicheometries of 0.66 Glu and 0.34 Arg per 70-kDa subunit. Their additional sequence was Glu-Gly-Ala-Glu-Asp-Ala. Position 3 differed from the analogous sequence determined by S.S. Taylor (Haas and Rosenberry, Anal. Biochem. 148, 154-162, 1985).

C. CLONING THE GENE FOR HUMAN AChE

We have initiated gene cloning by taking advantage of the sequence data and synthesizing oligonucleotide probes corresponding to the amino acid sequences. These probes are being used to screen cDNA libraries by colony hybridization (Grunstein and Hogress, PNAS 72, 3961-3965, 1975).

I. Synthesis of oligonucleotide probes

Several DNA probes containing a mixture of oligonucleotides that represent all possible codon combinations for small portions of the amino acid sequence of AChE have been synthesized:

Probe 1: 17 mer corresponding to peptide T7:

Amino acid		Pro	—	Asn	—	Gly	—	Pro	—	Try — Ala
Probe	3'	GGG A/C/T	—	TTG A	—	CCG A/C/T	—	GGG A/C/T	—	ACC — CG 5'

Probe 2: 14 mer corresponding to peptide T11:

Amino acid		Gly	—	Val	—	Pro	—	Gln	—	Val
Probe	3'	CCG A/C/T	—	CAG A/C	—	GGG A/C/T	—	GTC T	—	CA 5'

Probe 3: 13 mer corresponding to peptide T7:

Amino acid		Thr	—	Val	—	Gly	—	Met	—	Gly
Probe	3'	TGG A/C/T	—	CAG A/C	—	CCG A/C/T	—	TAC	—	CC 5'

Probe 4: 17 mer corresponding to peptide T10:

Amino acid		Gln	—	Try	—	Pro	—	Pro	—	Tyr	—	Thr					
Probe	3'	GTT/C	—	ACC	—	GGG/A/C/T	—	GGG/A/C/T	—	ATG/A	—	TG	5'				

Additional oligonucleotide probes (see Proposed Research) are now being prepared.

II. K562 cDNA libraries

cDNA libraries of the K562 human erythroleukemia cell line prepared in pBR322 and pUC 8 were obtained from Dr. Giovanni Rovera, Wistar Institute (Watt et al., Nature 303, 725-728, 1983).

c-myc positive control: A 17 base long oligonucleotide probe (5' CGG-CAA-CGA-CGA-GAA-CA 3') was synthesized according to the nucleotide sequence reported for c-myc gene by Watt et al. (Nature 303, 725, 1983). Using this probe strong positive signals were detected with both the K562 cDNA libraries (K562-pUC8 and K562-pBR322), indicating that the experimental protocol used was appropriate for hybridization with a 17 base long oligonucleotide probe. Based on this observation, AChE probes 1 and 4 (both 17 base long) were used to screen the cDNA libraries.

AChE Probes: Approximately 400,000 clones of each library were screened twice. Positive clones were not detected with probes 1 and 4.

III. Human muscle cDNA library

The human muscle cDNA library in pBR322 was obtained from Dr. L. Kedes, Stanford University (Gunning et al., Mol. and Cell Biol. 3, 787-795, 1983). This cDNA library was prepared as described by Okayoma and Berg (Mol. and Cell Biol. 7, 161-170, 1982) which optimizes conditions for the reverse transcription of mRNA and res[illegible]

in cDNA covalently linked to the plasmid vector and a high yield of full-length cDNA clones. This library is currently being tested with each of the 4 probes prepared from tryptic peptides. Preliminary evidence suggests the possible presence of cDNA clones (see Proposed Research).

D. STUDIES USING MONOCLONAL ANTIBODIES

A large number of immunizations with AChE and a putative active site peptide have been performed to obtain monoclonal antibodies directed at enzyme active site(s) and to prepare anti-idiotype immunoglobulins that mimic the enzyme in substrate binding. This phase of the program has been slow due to a low frequency of antibodies that inhibit enzyme activity. However, we now have obtained 2 such antibodies and are in the process of characterizing the mechanism of their action. In addition, we have prepared about 20 other clones with antibodies that bind to AChE without inhibiting enzyme activity.

I. Immunizations

a. AChE - Three different types of immunization protocols have been used for developing monoclonal antibodies (see Methods for details):

i. Conventional, Fambrough et al. (PNAS 79, 1078-1082, 1982).

ii. High specific efficiency, Cianfriglia et al. (Hybridoma 2, 451-457, 1983).

iii. Splenic, Thorpe et al. (Hybridoma 3, 381-385, 1984).

b. Consensus active site peptide - A peptide synthesized from the consensus sequence of serum butyrl cholinesterase and torpedo electric organ AChE was obtained from Dr. H. Soreq, Weizmann Institute. The reported sequence of this peptide was as follows: Val-Thr-Leu-Phe-Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser-Val. This peptide was conjugated to lipopolysaccharide and to keyhole limpet hemocyanin and used as immunogen. Hybridomas prepared from these immunizations were positive with the peptide but negative against AChE in solid phase radioimmunoassay and did not inhibit enzyme activity.

II. Monoclonal Antibodies

A large number of monoclonal antibodies that bind AChE have been obtained. As a representative sample, we have cloned over 20 of these for their potential interest (Fig. 7).

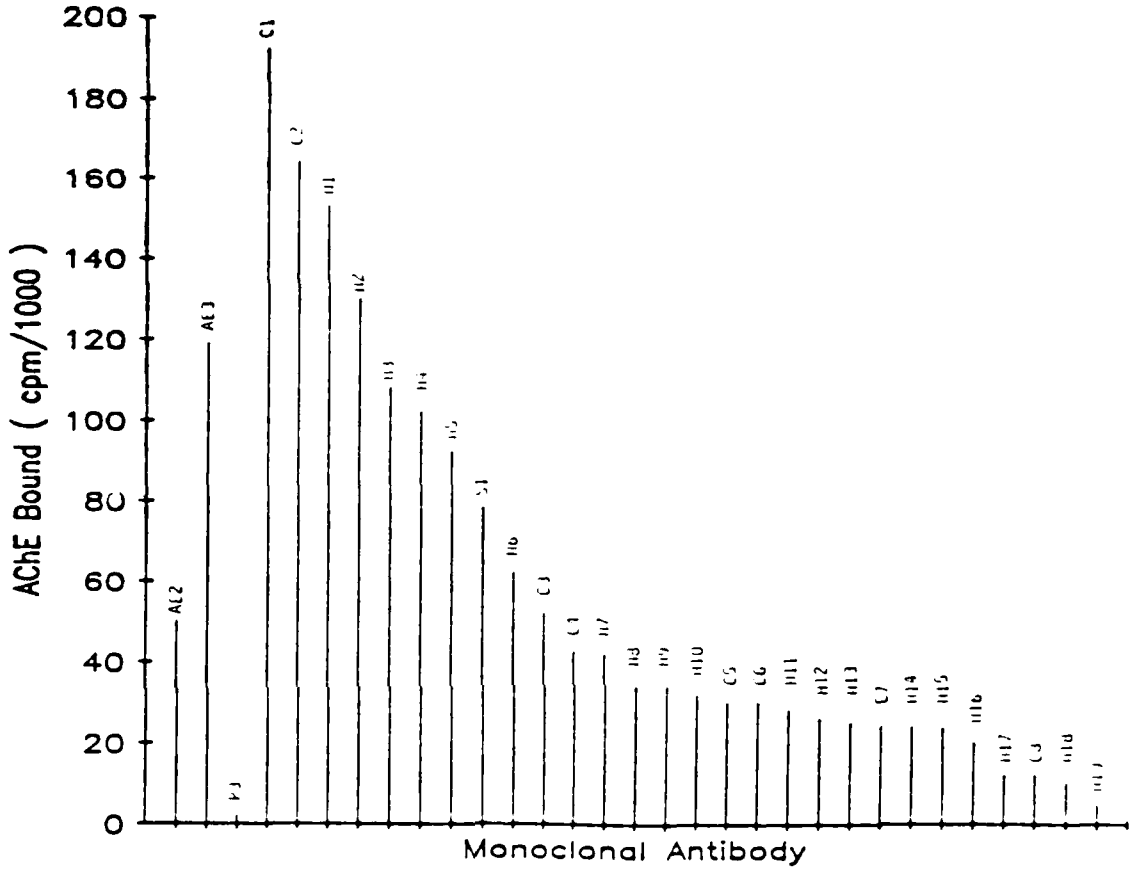

Figure 7: Monoclonal antibody binding to AChE. Culture supernatant (50 µl) was dispensed into wells of Dynatech polystyrene 96 well microtiter plates containing 5 µg goat anti-mouse IgG antibody adsorbed to the plate. After 1 hr the supernatant was removed, and 4 percent normal mouse serum (50 µl) was added to block excess goat anti-mouse IgG combining sites. ^{125}I AChE (10 ng, 10^5 cpm) was then added for 30 min, and the excess removed by washing with 1 percent BSA/PBS. Specifically bound AChE was taken up in 0.1 ml 2 M NaOH after incubation at 70°C for 15 min., and counted in a gamma counter. C = conventional; H = high efficiency; S = splenic immunization.

III. Antibodies That Inhibit AChE Activity

Two antibodies, H10 and H12, have been found to inhibit AChE activity (Figure 8). Both antibodies gave greater inhibition than observed with the AE2 antibody in assays with crude supernatants.

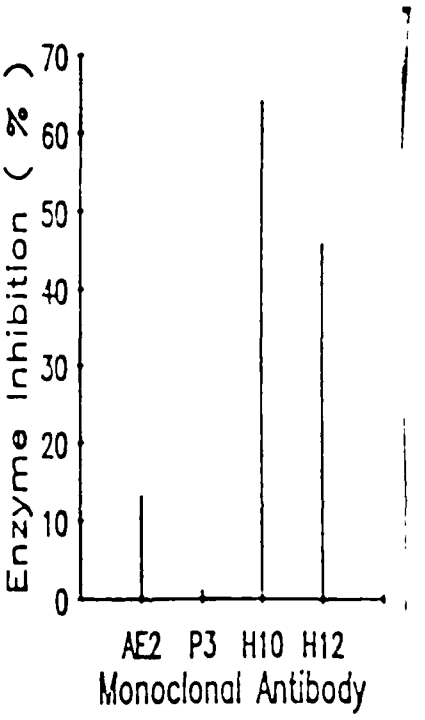

Figure 8: Inhibition of AChE activity by monoclonal antibodies. AE3 supernatant (50 µl) was used as in the RIA, followed by 10 ng unlabeled AChE. Next 50 µl of monoclonal antibody was added for 30 min and removed by washing. Enzyme activity was then analyzed by the addition of colorimetric agent and substrate (Ellman et al., Biochem. Pharm. 7, 88-95, 1961). AE2 = Fambrough isolate; P3 = control myeloma supernatant; H10, H12 = new monoclonal antibodies as described above.

IV. Characterization of Monoclonal Antibody Inhibition of AChE

Inhibition of AChE by the AE-2 antibody has been analyzed. Similar studies have been initiated with the two new inhibitory antibodies, H10 and H12.

Plan of study: Characterization of the inhibitory activity was carried out by use of a variety of enzyme inhibitors, measuring the effect of these agents on ^{125}I-enzyme binding to different monoclonal antibodies. Hydrolysis of acetylcholine by AChE involves both an electrostatic attraction between the choline moiety of acetylcholine and the anionic site or sites of the enzyme, and between the carboxyl group of acetylcholine and the esteratic site of the enzyme. Enzyme binding of the carbamyl ester neostigmine and related compounds is similar to the binding of acetylcholine, whereas other enzyme inhibitors bind primarily either to anionic sites (e.g. edrophonium) or to the esteratic site (e.g. DFP). We have studied the ability of these enzyme inhibitors to block the binding of purified AChE to the monoclonal antibody AE-2. The monoclonal antibody AE-4, which does not inhibit enzyme activity, was used as control for non-specific effects of the inhibitors on antibody binding. Under the conditions employed, antibody was present in excess and ^{125}I-AChE bound by monoclonal antibodies was proportional to the amount of ^{125}I-AChE added.

Inhibition of AChE by AE-2: Significant inhibition of the enzyme was observed with the AE-2 monoclonal antibody (Fig. 9). The maximum inhibition of enzyme activity by this antibody was about 50 percent.

Effect of neostigmine: Neostigmine was found to inhibit the binding of AE-2 by 20 to 50 percent at concentrations between 5 and 15 mM (Fig. 10). Inhibitor concentrations of 1 mM or less were not

inhibitory for antibody binding even though they represented 10^6-fold molar excess to the enzyme and blocked greater than 95 percent of enzyme activity. The requirement for a high concentration of neostigmine can possibly be attributed to a high affinity of the antibody for its epitope compared to the affinity of the enzyme for the pharmacologic inhibitor.

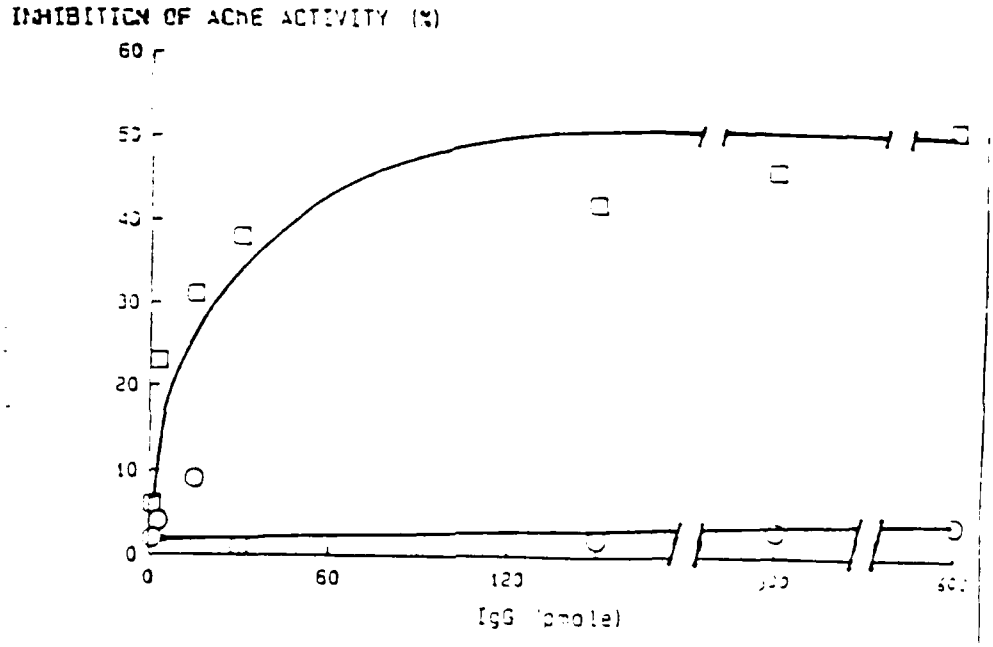

Figure 9: Effect of monoclonal antibodies on activity of purified acetylcholinesterase. AE-2 (□), AE-4 (○).

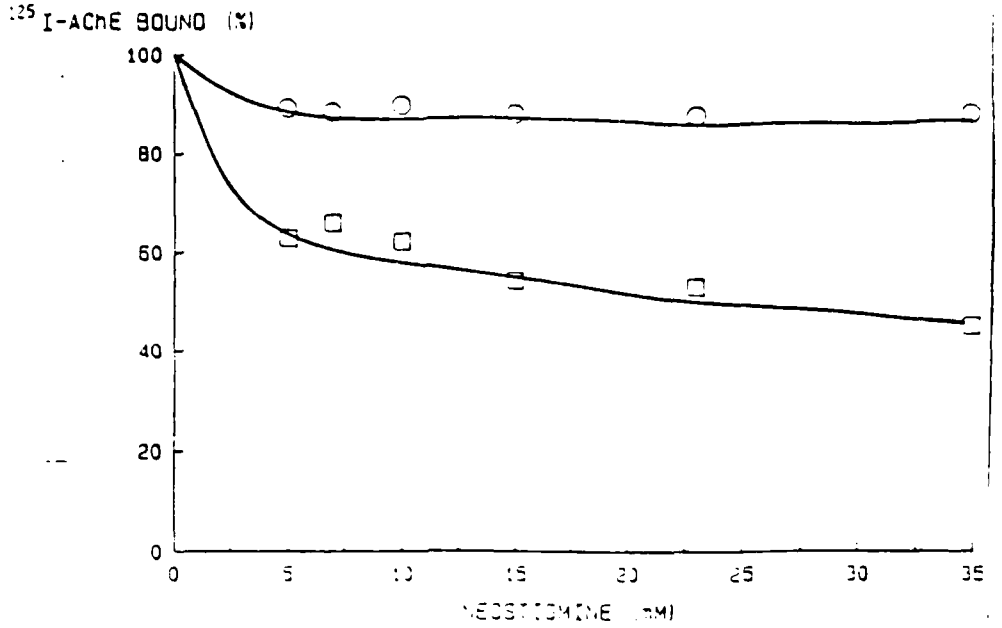

Figure 10: Effect of pre-incubation of acetylcholine with neostigmine on the binding of monoclonal antibodies to the enzyme. AE-2 (□), AE-4 (○).

BW 284C51: A similar effect was found for the compound BW 284C51, or 1,4 bis (allyldimethyl-ammonium-phenyl)-pentane-3-one-dibromide (Fig. 11). This compound binds reversibly to anionic sites but also affects the esteratic site, thus blocking the binding of DFP if BW284C51 is pre-incubated with the enzyme. It was found to be active at somewhat lower concentrations than neostigmine, i.e. at less than 1 mM, and showed up to 70 percent inhibition of enzyme binding to the antibody at the upper limit of concentrations (12 mM) giving a specific effect on AE-2 as compared to AE-4.

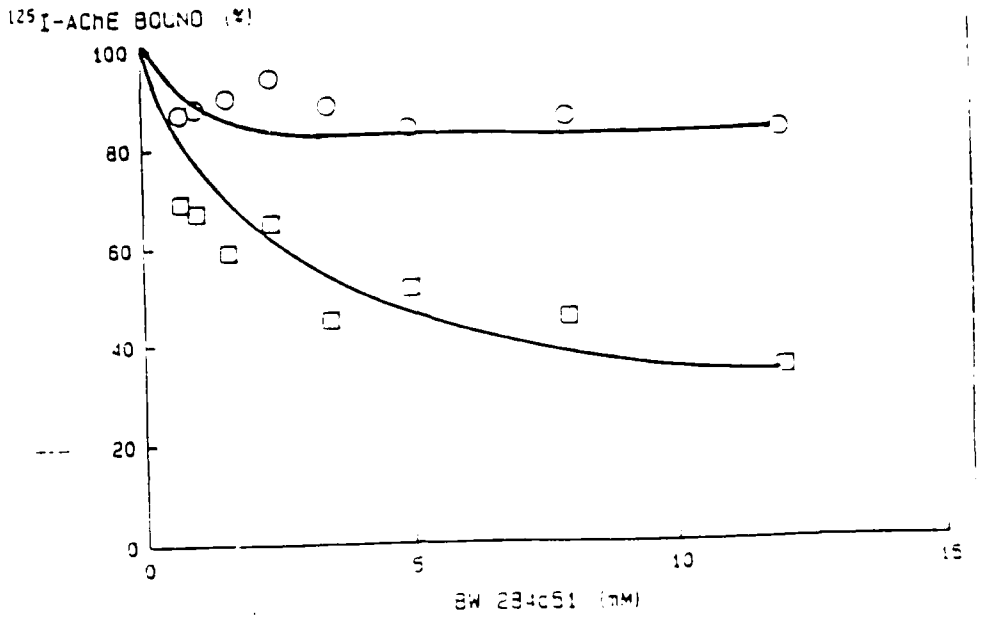

Figure 11: Effect of pre-incubation of acetylcholine with BW 284c51 on the binding of monoclonal antibodies to the enzyme. AE-2 (□), AE-4 (○).

DFP: The effect of drugs binding to or obstructing both anionic and esteratic sites was compared to the effect of agents which bind to either site alone. Pre-incubation of ^{125}I-AChE with the irreversible, esteratic site-binding inhibitor DFP failed to inhibit the binding of either AE-2 or AE-4 to the enzyme; in fact, a slight enhancement of binding appeared to take place with both antibodies.

Edrophonium: By contrast, when ^{125}I-AChE was pre-incubated with 5-50 mM edrophonium, a predominantly anionic site-binding compound, the binding of AE-2 but not AE-4 was inhibited by up to 50 percent (Fig. 12).

These data are consistent with a model in which the monoclonal antibody AE-2 blocks enzymatic activity by competing with the substrate for anionic sites or some related site.

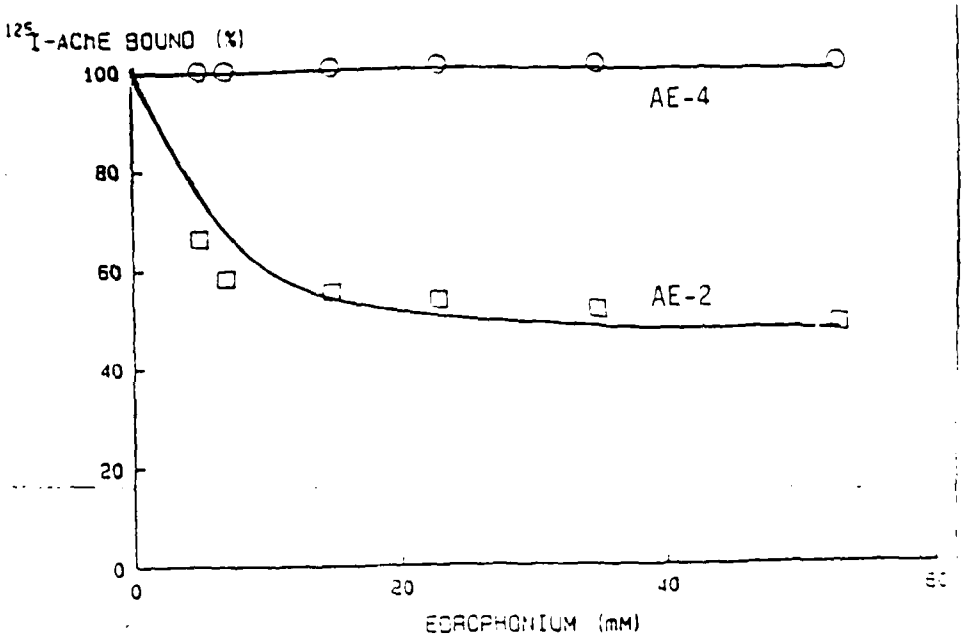

Figure 12: Effect of pre-incubation of acetylcholine with edrophonium on the binding of monoclonal antibodies to the enzyme. AE-2 (□), AE-4 (○).

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